REMARKS

I. Introduction

As an initial matter, applicants wish to thank Examiner Yu, and her supervisor, Examiner Eyler, for the courtesies extended by them during a February 17, 2004 interview. The present amendment reflects the contents of that interview.

Receipt is acknowledged of a final office action dated November 25, 2003. In the action, the examiner rejected claims 3, 4, 20 and 21 as allegedly indefinite and anticipated by Leippe *et al.* or Andra *et al.*, and claims 1-5, 8, 9 and 22 for obviousness reasons. The examiner also rejected claims 1-5, 8, 9, and 20-22 for double patenting and claims 3, 4, 20 and 22 for formality reasons. In the action, the examiner indicated that the double patenting rejection will be held in abeyance.

II. Status of the Claims

In this response, applicants amend claims 1 and 3 and add new claim 23. Support for the amended claims can be found throughout the specification, and on page 13 and in originally filed claims 1 and 3, in particular. Support for new claim 23 can be found in originally filed claim 5.

Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested. Upon entry of this amendment, claims 1-5, 8, 9 and 20-23 will be under examination.

III. Claim Objections

The examiner objected to claims 3, 4, 20 and 22 for allegedly failing to further limit the subject matter of a previous claim. In the interest of expediting prosecution, applicants have amended claim 1 to more clearly recite the presently claimed subject matter. Based on our February 17th interview, applicants believe that this amendment addresses the examiner's concerns.

IV. Rejection of the Claims Under 35 U.S.C. § 112, 2nd paragraph

Claims 3, 4, 20 and 21 were rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness. Office Action at page 3. Applicants respectfully traverse this ground for rejection.

Applicants trust that presently amended claims 1 and 3 alleviate the examiner's concerns.

V. Rejection of the Claims Under 35 U.S.C. § 102(b)

Claims 3, 4, 20 and 21 were rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by either Leippe *et a*l. or Andra *et al.* Office Action at page 5. Applicants respectfully traverse this ground for rejection.

The Examiner asserted that the "claims are interpreted as unmodified amoebapore peptide . . ." Office Action at page 5.

As previously argued, claims 3 and 4 are not drawn to an unmodified cytotoxic peptide since the claims recite that the cytotoxic peptide has "at least one lysine residue bound via a peptide bond to at least one amino acid via the ϵ-amino group of said lysine residue." Nevertheless, applicants have amended claim 1 to more clearly recite the modification in the claimed proctytoxin. This amendment renders the anticipation rejection moot.

VI. Rejection of the Claims Under 35 U.S.C. § 103

Claims 1-5, 8, 9, and 22 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over Leippe et al., *PNAS, USA,* 91:2602-2606 (1994) ("Leippe"), or Andra et al., *FEBS Letters, 385*:96-100 (1996), and further in view of Pinto *et al., The Prostate J., 1(1)*:15-26 (Jan/Feb 1999) ("Pinto"), Rivett *et al.*, WO 97/33908 ("Rivett"), and Liu *et a.l, Endocrinology, 104*(4):962-966 (1979 ("Liu") (abstract only). Office Action at pages 5-9. Applicants respectfully traverse this ground for rejection.

A. The Examiner's Basis for the Rejection

The examiner believes that it would have been obvious, at the time the claimed invention was made, to make "[a] procytotoxin comprising amoebapore H3 domain modified by linking two gamma linked glutamates through [an] epsilon group of the C-terminal lysine in order to inactivate the cytotoxic amoebapore H3 domain until it reaches target cells, namely prostate cancer cells expressing PSMA [,] which cleaves the gamma linked glutamates and selectively kills prostate cancer cells instead of causing havoc to [the] entire body of the person who has prostate cancer." Office Action at page 8. Applicants respectfully disagree.

B. Summary of the Cited References

Leippe describes the pore-forming peptide amoebapore. The sequence of amoebapore is given (page 2603), and the pore-forming properties of amoebapore are described (pages 2603-2604). Leippe also teaches that the activity of amoebapore can be enhanced by the addition of positively charged residues to the C terminus of the H1 peptide. *See e.g.*, pages 2603-04 ("[O]f the four synthetic peptides representing the helical structural elements of amoebapore, H3 exhibits the most pronounced activity in all biological assays employed; and (iii) the marginal biological activity of H1 can be greatly enhanced by attachment of a stretch of residues with a positive net charge."; and ". . . positively charged residues are considered critical for the lytic function of peptides.")

Andra describes analyzing the relationship between the structure and function of amoebapores by preparing 15 synthetic peptides of 24-25 residues based on the premise that the third helix is the membrane-penetrating domain and that charged residues are significant for activity. *See* the Abstract. The reference notes that all of the synthetic peptides exhibited pore-forming activity. Thus, none of the described peptides were non-lytic. Andra at page 98.

Pinto describes the full-length cDNA sequence, and the predicted amino acid sequence, for prostate specific membrane antigen (PSMA). This reference also reports the discovery that PSMA exhibits folate hydrolase activity as demonstrated by using as a substrate a modified methotrexate compound. Methotrexate was modified by adding a tri-

gamma-glutamate to the compound. *See* page 19 of Pinto. Methotrexate is a small molecule anticancer agent; it is not a peptide.

Rivett refers to a peptide having lytic activity, along with methods of activating and inactivating the lytic activity. Methods of inactivating the lytic activity of a peptide include adding an amino acid sequence or other group at the amino terminal of an otherwise lytically active peptide comprising an amphipathic alpha-helix (page 4, lines 24-27). This does not suggest or teach the method or compositions of the claimed invention, either alone or in combination with the other cited references.

Liu refers to a comparison of the effect several gonadotropin-releasing hormone (GnRH) analogs have on regulating the synthesis and release of luteinizing hormone. The GnRH analogs include an GnRH agonist having the sequence (D-Lys6, epsilon-polyglutmate)GnRH. The abstract concludes that GnRH analogs have similar effects on luteinizing hormone synthesis and release.

C. A Motivation to Combine the Cited References is Lacking

As described in more detail below, the art does not establish: (1) some suggestion or motivation to modify the reference or to combine reference teachings, (2) a reasonable expectation of success, and (3) that the prior art references, when combined, teach or suggest all the claim limitations to establish a *prima facie* case of obviousness. *See* MPEP §2143 (Aug. 2001). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Vaeck*, 947 F.2d 488, 493 (Fed. Cir. 1991).

D. None of the Cited References Teach or Suggest Modifying a Peptide as Claimed by the Present Invention

During the February 17th interview, the examiner indicated that Pinto teaches adding gamma glutamates to make a prodrug and therefore, it would have been obvious to add gamma glutamates to a cytotoxic peptide and make the procytotoxin of the claimed invention.

1. In Contrast to Small Molecules, Which are the Subject Matter of Pinto, the Activity of Peptides is Dependent Upon the Secondary and Tertiary Structure of the Peptides

Foremost, the present invention relates to modifying peptides, and Pinto describes modifying small molecules. This is significant, as factors that affect the activity of small molecules are very different from those that affect the activity of a peptide. Specifically, modifying the sequence of a peptide, as required by the claimed invention, can change the conformational structure of the peptide and thereby alter the activity of the peptide. This risk is not evident with small molecules, as the activity of small molecules is not dependent upon conformational structure.

A striking characteristic of peptides is that they have well-defined three dimensional structures. The strong tendency of hydrophobic amino acid residues to flee from water drives the folding of soluble peptides. A stretched-out or randomly arranged polypeptide chain is devoid of biological activity. This is because the function of a peptide arises from conformation, which is the three dimensional arrangement of atoms in a structure. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, p. 1-41 (W.H. Freeman & Co., NY, 1988). Amino acid sequences are important because they specify the conformation of peptides. *Id.*

Peptides have several different defined structures, including a primary, secondary, and tertiary structure. The primary structure of a peptide is generally the amino acid sequence of the peptide and the location of disulfides. *See e.g.*, L. Stryer, *Biochemistry,* p. 31. Secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. Examples of these steric relationships are structures known as an alpha helix, a beta pleated sheet, and a collagen helix. *Id.* Tertiary structure refers to the spatial arrangement of amino acid residues in a peptide or polypeptide that are far apart in the linear sequence.

Proteins, comprising multiple polypeptide chains, also have a quaternary structure, which refers to the spatial arrangement of the polypeptide subunits and the nature of their contacts. *Id.*

The correlation of conformational structure with activity for peptides is also described by Leippe. This reference teaches that the activity of amoebapore is completely dependent upon the tertiary structure of the peptide, as loss of the structure correlates with a loss in

activity. *See* page 2605 ("In the intact amoebapore molecules the helices are embedded within a rigid tertiary structure constrained by three disulfide bonds which do not allow a major conformation rearrangement. . . . Reduction of the disulfide bonds results in complete loss of activity . . .").

Given that peptides have such complex conformational structures, which correlate with activity, modification of a small molecule does not teach or suggest a similar modification to a peptide with any expectation of success that such a modified peptide will possess the desired activity. At best, such a reference would provide a motivation to *try* such a modification, but *obvious to try is not the standard for obviousness.* Thus, Pinto fails to provide motivation to one of skill in the art at the time the claimed invention was made to modify peptides, as claimed by the present invention.

2. Pinto Teaches a Different Modification Point as Compared to that Encompassed by the Claimed Invention

Pinto teaches adding γ-glutamates *at the end* of the small molecule methotrexate so that PSMA can sequentially remove the glutamate residues. However, simply adding gamma glutamates to the end of a cytolytic peptide would not necessarily render the cytolytic peptide inactive.

In other words, the modification to the claimed procytotoxin has to be between the ε-amino group of at least one lysine residue and at least one amino acid. Pinto does not teach or suggest this modification. Moreover, the specific modification of adding a γ-glutamate moiety to a lysine residue to charge neutralize the cytolytic peptide and inhibit folding is also not taught or suggested by any of the other cited references. Thus, Pinto fails to provide motivation to one of skill in the art at the time the claimed invention was made to modify peptides, as claimed by the present invention.

3. Methotrexate, the Active Compound of Pinto, is Significantly Different from the Lytic Peptide of the Claimed Invention

In addition to small molecules and peptides behaving differently in general, methotrexate and lytic peptides themselves are vastly different. They: (i) vary significantly in size, (ii) fall within different classes of compounds, and (iii) have distinctly different

modes of action; while methotrexate acts intracellularly, lytic peptides act on the surface of the target cell and bore holes into the cell membrane.

Accordingly, one of skill in the art would not have been motivated to apply the teachings related to the small molecule methotrexate to a peptide with any reasonable expectation of obtaining a peptide having the desired activity. The Pinto reference is, in fact, non-analogous art.

4. The Mechanism of Action for Methotrexate is not Equivalent, or Relevant, to that Encompassed by the Claimed Invention

As noted above, methotrexate acts *intracellularly* while the lytic peptides encompassed by the claimed invention act on the surface of the target cell, or *extracellularly*, to bore holes into the cell membrane.

The examiner's argument is that Pinto teaches adding gamma glutamates to affect toxicity of a compound. In its broadest sense, this is true only in that Pinto demonstrates that methotrexate is less toxic to all cells **because it cannot enter any cell in its glutamated form** and exert its activity *intracellularly*. However, as described in Pinto, the toxicity of poly-γ-glutamated methotrexate is blocked in cells lacking PSMA, not that $MTXglu_3$ is less toxic itself. *See*, Pinto at 22. Indeed, Pinto teaches that "once deglutamated and transported into cells, folate derivatives [such as methotrexate] are re-glutamated and thus are retained intracellularly." (*Id.*). Thus, poly-γ-glutamated methotrexate, once inside a cell, is still able to kill a cell. The gamma glutamates only act to inhibit transport across the plasma membrane and not to render the methotrexate inactive. In other words, γ-glutamated methotrexate is *not* an inactivated form of methotrexate. Rather, $MTXglu_3$ is still in an active conformation but merely has an attached moiety that acts as a physical barrier to cell entry.

This is in contrast to the procytotoxins of the present invention, which comprise a cytolytic peptide that is inactivated by the addition of γ-glutamates. The proctyotoxins are unable to fold into a pore forming conformation correctly and therefore, are not lytically active. In other words, the procytotoxin will not insert into the membrane of a target cell because it will not organize into a pore-forming unit until the modifying amino acid is removed. Thus, the addition of γ-glutamates to the cytolytic peptide act to neutralize charges

and prevent folding of the cytolytic peptide into an active conformation. This is not taught or suggested by any of the cited art.

E. Leippe, Either Alone or in Combination, Does Not Teach or Suggest the Claimed Invention

The examiner also relied on Leippe, which allegedly teaches which residue in amoebapore should be bound via a peptide bond to at least one amino acid via its ϵ-amino group.

As noted above, Leippe describes the pore-forming peptide amoebapore and modifying the peptide, which can change the three dimensional conformation of the peptide, can result in undesired consequences. The reference further describes that adding a positive net charge to the C terminus of helix 1 (H1) can increase pore-forming activity but does not describe the addition of amino acids to any particular residue, let alone to the side chain of a lysine residue, to decrease peptide activity. Indeed, nothing in this reference teaches or suggests that lytic peptides can be successfully modified as claimed to render the peptides inactive, followed by activation when desired.

F. Liu, Either Alone or in Combination, Does Not Teach the Claimed Invention

According to the Examiner, Liu allegedly describes that γ-glutamate linkages are known in the art.

Liu describes gonadotropin-releasing hormone (GnRH) analogs, including the analog (D-Lys6, epsilon-polyglutmate)GnRH. Nothing in this reference teaches or suggests the claimed modified peptides, nor that such modification can affect the lytic activity of the peptides. In fact, Liu describes poly-γ-glutamated GnRH as an agonist.

For at least these reasons, withdrawal of this ground for rejection is respectfully requested.

CONCLUSION

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and arguments.

It is respectfully urged that the present application is now in condition for allowance. Early notice to that effect is earnestly solicited.

The examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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By _____

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